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ORIGINAL ARTICLE

Comparison of various methods for DNA extraction from human isolated paraffin-embedded hydatid cyst samples

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Abstract Successful molecular research with reliable results depends on achieving significant and uniform amounts of genomic DNA from the parasite as the first and most basic step. Therefore, selection of an appropriate method that minimizes damage to the DNA of the parasite, is very important. In this study, we are going to describe a method that can extract DNA from human isolated paraffin-embedded hydatid cysts with a high quality and quantity. Formalin fixed and Paraffin-embedded hydatid cyst samples isolated from human lung and archived in the pathology laboratory were used for this purpose. Several sections of the paraffin blocks were prepared with 5 micron thickness and DNA were extracted by three different methods including; modified boiling, commercial kit and the method described by Larissa A. Pikor et al. The obtained DNA were evaluated by Nanodrop in terms of the yield of DNA and possible contaminations. To compare the quality of DNA prepared, *cox*1 region was amplified using specific primers. It was found that the DNA extracted by modified boiling had the lowest rate of contamination and the best electrophoretic band on the gel, compared to other two performed methods. Considering the findings of this study, this simple and high throughput DNA extraction method with high yield and quality can be recommended for extraction of DNA from formalin fixed and paraffin-embedded hydatid cysts.

Keywords DNA extraction · Paraffin-embedded · Hydatid cysts

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Introduction

Hydatidosis is one of the most important parasitic diseases which is caused by the larval stage (hydatid cyst) of *Echinococcus granulosus* (Thompson 2008; Moshfe et al. 2019). Canids are considered as the definitive hosts of this metacestoda and in most areas, stray dogs and herds are infected with the adult form of the parasite (Gholami et al. 1999; Ansari-Lari 2005; Dalimi et al. 2002). Humans and herbivores such as sheep, cattle and goats are intermediate hosts of the parasite. Dogs excrete eggs and the intermediate hosts acquire infection through ingestion of the eggs (Romig et al. 2017). Areas where wastes of slaughter animals are disposed of in an unsanitary manner so that eaten by stray dogs or herds, can maintain the life cycle of the parasite; the incidence of human hydatidosis is mostly related to such areas (Gholami et al. 2011).

It is known that *E. granulosus* has different genotypes, which can affect life cycle pattern, epidemiology and pathogenicity of hydatid cysts (Bowles et al. 1992; Khosravi et al. 2012; Sharbatkhori et al. 2009; Barazesh et al. 2019), as it is proved that some of the strains are more virulent in humans and also tend to be specific organs of the host body (Gholami et al. 2011). Therefore, determining the dominant genotypes of the parasite in different regions and subsequently, providing appropriate and

effective therapeutic strategies is of particular importance (Barazesh et al. 2019; Kheirandish et al. 2018; Ahmadi and Dalimi 2006).

The use of surgically removed and formalin-fixed hydatid cyst specimens which have been archived as paraffin-embedded blocks in pathology laboratories, can be a good way to determine the dominant genotypes of this parasite in different regions (Gholami et al. 2011). In this way, it can be easily and at a low cost provide a rich archive of samples collected over several years (Tafvizi and Assareh 2015). But one of the major drawbacks of using formalin fixed and paraffin-embedded tissue samples compared to fresh ones is that DNA may be broken during the extraction process into smaller fragments. This is because of the strong links between protein and DNA that must be broken during the extraction step, which may cause DNA fragmentation and decrease the quality of subsequent molecular studies (Tafvizi and Assareh 2015; Weiss et al. 2011).

Since successful molecular and genetic research with reliable results, requires the acquisition of significant and high-quality quantities of parasitic genomic DNA as the first and most essential step, choosing an appropriate approach that minimizes parasite DNA damage, while still being easy, functional and low-cost, is important.

Various methods can be used to extract parasitic DNA from specimens. Hazrati Tappeh et al. (2012) used four different methods; Glass beads, Boiling, Crushing and Commercial DNA extraction kit to extract parasitic DNA from collected sheep and cattle hydatid cysts and compared their efficiency. Other methods used for this purpose include Freezing–thawing and Mechanical grinder techniques (Al-Azawi et al. 2014) and also Phenol/chloroform method (Hizem et al. 2016).

In the previous study, we used and compared various methods for the extraction of DNA from pulmonary and liver hydatid cyst protoscolices isolated from Cattle and sheep (Barazesh et al. 2018). In the following, we are going to describe another method which can extract DNA from paraffin-embedded and formalin fixed human isolated hydatid cyst samples with better quality and quantity.

Materials and methods

Sample preparation and extraction of genomic DNA

About 5 Paraffin-embedded and formalin fixed human isolated pulmonary hydatid cysts and archived in the pathology laboratory were used for this purpose. Multiple sections of 5 micron thickness were prepared by a microtome from paraffin blocks and DNA was extracted in the following ways:

Modified boiling method

At this stage, minor modifications were made to the method that Safaei et al. (2002) performed in their research on skin biopsies suspected of cutaneous leishmaniasis. Briefly, 25 mg of the tissue slices was poured into a microtube and mixed thoroughly with 1 ml of xylene and incubated at room temperature (RT) until complete dissolution of paraffin in the sample. Then centrifuged at 14,000 rpm for 10 min and supernatant was removed. This step was repeated again in order to completely deparaffinize the sample. About 0.5 ml of absolute ethanol was added to the tissue sediment and incubated at RT for 5 min and this step was repeated again. One hundred microliters of acetone was added and incubated overnight at RT. After that, the tissue was thoroughly crushed with a sterile rod and boiled for 15 min after addition of 100 µl of TE buffer (Tris–HCL 10 mM + EDTA 1 mM). After cooling the sample and centrifuge at 14,000 rpm for 15 min, the supernatant was harvested as a DNA source and stored at − 20 °C (Safaei et al. 2002).

Using commercial kit

According to the kit manufacturer's protocol (YTA, Yekta Tajhiz Azma, Iran), in brief, 25 mg of tissue slices was exposed to 1 ml of xylene and incubated until complete dissolution of paraffin in the sample. After centrifugation at 14,000 rpm for 10 min, the supernatant was removed. Absolute ethanol was added to the sediment in two steps and centrifuged at maximum speed (18,000 rpm) for 3 min. Then incubated at 37 °C until complete evaporation of the alcohol in the samples. Subsequently, TG1 buffer and Proteinase K were added and incubated at 60 °C for 48 h and then 90 °C for 30 min. The buffer TG2 and absolute ethanol were added to the samples in the order and then were taken on column and after centrifugation at 18,000 rpm and washing with W1B and then Wash buffers, centrifuged again at 18,000 rpm. Finally, by adding of Elusion buffer, DNA was extracted and stored at − 20 °C.

The method described by Pikor et al. (2011)

Briefly, 800 µl of xylene were added to the tissue sections and shaked gently for 15 min to dissolve the paraffin. The samples centrifuged at max speed and supernatants were removed. After fully deparaffinization the samples by repeating xylene wash step again, rehydration was performed by adding serial concentrations of ethanol (100%, 70% and 50%, respectively), vortex and centrifuge at 14,000 rpm for 5 min and finally, the sediments were air-dried for 5 min. In order to remove destructive proteins and enzymes, lysis buffer containing SDS as well as proteinase

K were used and the samples incubated at 56 °C until were fully dissolved. Then in two steps, saturated phenol buffer was added to the lysed tissue and centrifuged at 14,000 rpm. The supernatant containing the DNA was collected and frozen until further tests (Pikor et al. 2011).

Quantification of DNA

All three extracted samples were evaluated for DNA concentration along with possible carbohydrate and protein contamination by Nanodrop (Thermo, USA).

PCR and gel electrophoresis

Pairs of primers JB3 (F) and JB4.5 (R) with sequences (5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′) and (5′-TAA AGA AAG AAC ATA ATG AAA ATG-3′) (Bowles et al. 1992) respectively, were used to amplify the *cox*1 region as a bit of the parasitic mitochondrial genome. Temperature pattern accustomed to amplify the genomic piece was set as follows: 1 × (5′ 95 °C) + 40 × (45″ 94 °C + 35″ 51 °C + 45″ 72 °C) + 1 × (10′ 72 °C).

The PCR products were electrophoresed on 1.5% agarose gel with TAE buffer and gel red and also the bands were visualized by UV Detector (Bio-Rad, USA).

Results

All extracted DNA samples in three different ways were evaluated by Nanodrop in terms of the amount of DNA concentration and possible carbohydrate and protein contamination. As shown in Table 1, the concentration of DNA obtained in the first method is 12 ng/µl which is not much different from the concentrations obtained during the second (11 ng/µl) and third (12 ng/µl) methods, but in terms of protein and carbohydrate contaminations, it had the lowest rate in the first method, compared to the other two methods used.

Figure 1 shows the electrophoretic bands of *cox*-1 genomic PCR products from three extracted DNA samples. Bands 1–3 related to the samples using modified boiling method, commercial kit and Larissa method, respectively, and band M is correspond to the molecular weight marker (Fig. 1).

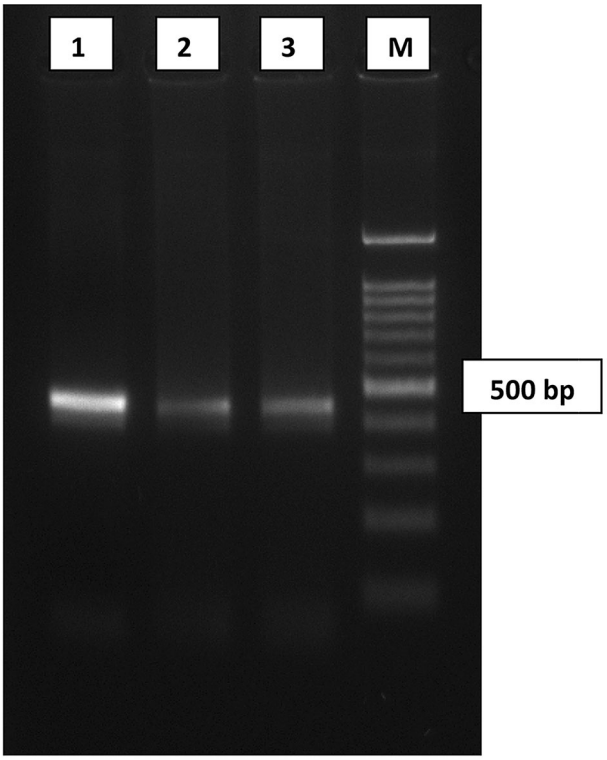

Fig. 1 Electrophoresis of PCR products using extracted DNA by different methods. Lane 1: samples extracted by modified boiling method; lane 2: samples extracted by the commercial kit; lane 3: samples extracted by Pikor et al. method; *M* molecular weight marker (in base pairs)

Discussion

Surgically removed and formalin-fixed hydatid cyst specimens and archived as paraffin blocks in pathology laboratories, are a rich treasure trove of specimens collected over many years which can be obtained valuable samples of DNA at the lowest cost. But one of the major drawbacks of using fixed tissue samples compared to fresh ones is that DNA may be broken during the extraction process into smaller fragments and decrease the quality of subsequent molecular studies (Tafvizi and Assareh 2015; Weiss et al. 2011).

The first method used in this study to extract DNA from paraffin-embedded human pulmonary hydatid cysts, was the boiling method with minor modifications in the procedure that Safaei et al. (2002) conducted in their research

Table 1 Comparison of obtained DNA from different methods resulted by Nanodrop

Methods	DNA concentration (ng/µl)	Protein contamination (260/280)	Carbohydrate contamination (230/280)
Modified boiling	12	1.8	1.7
Commercial kit	11	1.65	1.2
Pikor et al. method	12	1.5	1.45

on skin biopsies suspected of cutaneous leishmaniasis. Due to the fact that in the boiling method, there is a possibility of breaking the DNA during the extraction process and may creating smaller fragments and its negative impact on molecular studies (Tafvizi and Assareh 2015; Weiss et al. 2011), so it is recommended that this method be used for DNA fragments with less than 400 bp, because the large fragments are destroyed by boiling and the probability of successful amplification will be low. Moreover, it is better to use the samples that have not been prepared for more than 5 years (Sepp et al. 1994; Davoodi et al. 2009).

The results obtained with nanodrop and gel electrophoresis images show that the best results in DNA purity and reduced protein and carbohydrate contamination and also the best band was obtained using modified boiling method. In the past, paraffin-embedded samples have not been considered as suitable sources for molecular analysis because of the potential cross reactions between protein–protein and nucleic acid–protein that strongly affect the structure of nucleic acids, but with the discovery of protease digestive enzymes, it has been possible to access to healthy nucleic acid fragments that are suitable for a variety of molecular studies (Pikor et al. 2011; Tang et al. 2009). Even though paraffin embedded samples are not recommended for RNA studies, it is possible to extract RNA from such samples, either as paraffin blocks or as stained slides.

Different methods have been considered for extracting DNA from formalin-fixed and paraffin-embedded tissue samples and each of which has its disadvantages and advantages. In a study conducted in 2011 by Gholami et al. (2011) on paraffin-embedded human hydatid cysts with the aim of determining the genotypes of *E. granulosus*, they have used almost the same method as Larissa et al.; after deparaffinization, the samples were rehydrated using serial concentrations of ethanol and finally, they used lysis buffer containing SDS as well as proteinase K to digest destructive enzymes and proteins. The authors have considered the use of paraffin-embedded and formalin fixed human hydatid cysts to determine the genotype of *E. granulosus* and the results obtained indicate the existence of different genotypes of the parasite in different parts of Golestan province.

Except in hospitals located in hyperendemic areas for hydatid cysts, it is difficult to collect new human hydatid samples and it has been found that in most European countries and regions with very low prevalence of hydatidosis, paraffin-embedded human hydatid cysts blocks are the only specimens available for further investigation on the strains of this parasite. Other advantages of paraffin blocks are the ease of carrying samples as well as their appropriate storage in the laboratory (Schneider et al. 2008).

Therefore, considering the availability of archived paraffin-embedded specimens in most parts of the world and in view of the results of this study, it is recommended to use modified boiling method for DNA extraction and molecular studies of such specimens.

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Authors' contribution Designed the study: AB, SE; Performed laboratory affairs: AB, MR, SE; Imaging: SE, MF; Participated in writing the final paper: AB, MF, MR.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research was approved by the ethics committee of Bushehr University of Medical Sciences (Ethics Committee Code: 6053).

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